UNDERSTANDING THE ROLE OF IRON-SULFUR CLUSTERS IN THE EVOLUTION OF RNA POLYMERASE

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Abstract: DNA-dependent RNA polymerase (RNAP) is a multi-subunit enzyme which synthesizes RNA from a DNA template and is essential to all cells. Archaeal RNAP consists of 12-13 subunits and is similar to eukaryotic RNAP, but not bacterial RNAP, which consists of five subunits. Moreover, subunit D of RNAP from numerous archaea and the homologous Rpb3/AC40 subunits of RNAP from several eukaryotes contains a [4Fe-4S] ferredoxin-like domain predicted to bind one or two [4Fe-4S] clusters, consistent with archaeal and eukaryotic RNAP having a common ancestor. We hypothesize the ferredoxin-like domain was acquired to coordinate RNAP assembly and activity (i.e. transcription) with the metabolic state of the cell. Subunit D forms a heterodimer with subunit L, which is the first step in the assembly of RNAP. Interestingly, only RNAP from strictly anaerobic archaea is predicted to bind two [4Fe-4S] clusters, including the majority of methanogenic archaea (methanogens), whereas RNAP from other archaea and eukaryotes are predicted to bind a single [4Fe-4S] cluster or lack clusters entirely. We have previously demonstrated that subunit D of RNAP from *Methanosarcina acetivorans*, a member of the Methanosarcinales, contains two oxygen-labile [4Fe-4S] clusters, which impact the stability of the D-L heterodimer [1]. However, not all methanogens contain a subunit D predicted to bind two [4Fe-4S] clusters. For example, RNAP from *Methanobrevibacter smithii*, a member of the Methanobacteriales, contains a subunit D predicted to bind only one [4Fe-4S] cluster. To understand the importance of one or two [4Fe-4S] clusters to the assembly and activity of RNAP from different lineages of methanogens, the goal is to purify the D-L heterodimer from *M. smithii* and determine the number, type, and properties of the Fe-S clusters, as well as the impact of the clusters on the stability of the D-L heterodimer. A plasmid-based system was developed to co-express recombinant subunit D and subunit L in *Escherichia coli.* Preliminary data reveal that co-expression of D and L in *E. coli* results in the formation of a D-L heterodimer, whereas expression of D alone results in inclusion bodies. Purification of the *M. smithii* D-L heterodimer and reconstitution with iron and sulfide produced results consistent with the presence of a [4Fe-4S] cluster. These results indicate that subunit D of RNAP from *M. smithii* contains a single [4Fe-4S] cluster, supporting the hypothesis that some methanogens evolved to use a single cluster to potentially regulate the assembly and activity of RNAP.

References: [1] Lessner F. H., Jennings M.E., Hirata A., Duin E.C., Lessner D.J. 2012. *J Biol Chem* 287(22): 18510-18523